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Role of the Medial Septal Area in Regulating Prefrontal Theta Rhythm in Rats

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Abstract

Theta rhythms are electroencephalogram (EEG) waveforms between 4-12 Hz and are correlated with arousal, orientation, exploration, attention, learning and memory, motivational drives and emotions and movements. The last sixty years have been witness to a greatly increasing understanding of the underlying anatomical pathways and mechanisms necessary for theta rhythms. Today, it is well established that cells of the medial septal area (MSA) fire in a rhythmic bursting pattern to pace the theta rhythm in the hippocampus (HPC) and that lesioning the MSA abolishes theta rhythm in the HPC. However, comparatively little is known about the anatomy driving the theta rhythm of non-hippocampal areas, such as the prefrontal cortex (PFC). Therefore, this study examined whether the MSA also drives the theta rhythm in the PFC. Results indicated that selective infusions of the muscarinic receptor antagonist scopolamine into the MSA significantly decreased PFC theta power but had no effect on theta frequency. While this study has not been conducted before, these results coincide with other studies implicating the MSA as a widespread controller of theta power. Thus, it appears that the MSA affects PFC theta in the same manner as HPC theta with regards to both power and frequency.

Introduction

Theta is currently recognized as a quasi-sinusoidal, slow-wave field activity of up to 2 mV in amplitude with a frequency range of 4-12 Hz in rodents. Seventy-three years have elapsed since Saul and Davis (1933) first described the "action currents" they recorded from the hippocampus. Today, Green and Arduini's (1954) paper is viewed as the benchmark for the beginning of the intensive study of hippocampal theta rhythm. From that point on, theta researchers pursued two separate though not necessarily independent lines of research. One approach was to correlate theta with various cognitive and behavioral concepts. The other approach was to study the physiology of the theta rhythm; these physiological studies were concerned with the topography of theta within the hippocampal formation and the cellular mechanisms and afferent systems involved in theta generation (Bland, 1986). The majority of theta research has focused on examining the cognitive and physiological attributes of theta within the HPC system. However, more recent studies have branched out to examine theta activity in non-HPC sites such as the prefrontal cortex (PFC). While hippocampal theta and the mechanisms controlling it are well understood, extensive research still needs to be conducted on prefrontal theta rhythms.

Theta and cognition/behavior

C.H. Vanderwolf (1969) is credited with being the first to carry out studies correlating hippocampal theta with observable behavior. These studies, based on rats, resulted in his theory that theta activity is the electrical sign of activity in a forebrain mechanism which organizes or initiates higher (voluntary) motor acts. The ensuing years were witness to a spate of publications showing that hippocampal theta activity in a

variety of species was related to ongoing motor behavior. The bulk of these observations were reported for rats (Routtenberg & Kramis, 1968; Bland & Vanderwolf, 1972; Whishaw & Nikkel, 1975; Kramis & Routtenberg, 1977; Kramis & Vanderwolf, 1980; O'Keefe & Recce, 1993; Vanderwolf, 1969; Whishaw, Bland & Vanderwolf, 1972; Whishaw & Vanderwolf, 1973). Additional researchers have furthered these studies, suggesting that theta is intimately involved in the expression of goal-oriented behavior. Such studies have been supported structurally (Risold & Swanson, 1996) and through behavioral tasks such as water and radial-arm mazes (Bland, 1986). Since the majority of authors have regarded theta as an expression of certain behavioral activities which are ascribed to the hippocampus, theta has therefore been regarded as a "correlate" of arousal, orientation, exploration, attention, learning and memory, motivational drives and emotions and movements (Vinogradova, 1995).

Dietl, Dirlich, Vogl, Lechner and Strian (1999) reported that theta oscillations have, in particular, been associated with the encoding and retrieval of new information (Givens, 1996), the recognition of familiar stimuli (Burgess and Gruzelier, 1977), and learning in general. In their studies of the time course of stimulus-to-stimulus changes of theta activity under repetitive somatosensory stimulation, Dietl et al. (1999) observed that theta activity synchronizes with cognitive demands. Their main finding was a frontal midline activation in the theta band with the beginning of the stimulus train, which habituates during the subsequent stimulation cycles. Additional research has shown that a simple oscillatory neuronal network that incorporates such sustained activation can be used in short-term memory encoding (Lisman & Idiart, 1995).

Additional studies report that theta rhythms are heavily implicated in memory. For instance, it has been demonstrated that retention is improved by post-trial electrical stimulation of different structures such as reticular formation, hippocampus or septum, which induces hippocampal synchronization (rhythmic slow activity, RSA) after learning. Jensen and Tesche (2002) observed that theta oscillations generated in frontal brain regions play an active role in memory maintenance. Givens (1996) noted that task-relevant sensory stimuli elicited a synchronization, or resetting, of rhythmic activity in the HPC of rats performing a working memory task. This synchronization of theta rhythm provides a means by which environmentally relevant stimuli may become time-locked to waves of depolarization in the hippocampus so that the sensory representation can be enhanced and strengthened for later retrieval (Givens, 1996). Thus, effective memory encoding and retrieval depend heavily upon theta resetting.

Theta resetting is dependent upon the MSA, a region directly connected to the HPC (Partlo & Sainsbury, 1995; Shaban, 1970). Researchers have also observed that the septohippocampal pathway, or fornix, provides the HPC with a rhythmic input that drives the hippocampal theta rhythm (Givens, 1996). When septohippocampal activity is simultaneously recorded with behavior, disruption of the hippocampal theta rhythm reliably produces impairments in working memory. Given that working memory, but not reference memory, is critically dependent on rhythmic activity in the hippocampus, the resetting of rhythmic activity may likewise be related to processing stimuli for working memory, and would not be necessary in a well-rehearsed reference memory task. The results suggest that accurate hippocampal processing of stimuli for working memory may involve a phase-locking of theta activity to stimulus onset. This mechanism allows septal

and entorhinal inputs to the dentate to become temporally coupled under behaviorally-relevant conditions. The precise activation of MSA afferents at the time that entorhinal inputs carrying sensory signals arrive in the HPC may lead to synaptic potentiation of the signals (Klimesch et al., 2001). Given the importance of theta activity to a variety of cognitive/behavioral processes, many researchers turned their attention to examining the underlying anatomical mechanisms behind theta activity.

Theta Anatomy

Theta can be recorded from many regions of the limbic cortex including the areas of the HPC, medial forebrain bundle (MFB), MSA and dorsomedial hypothalamus (DMH) (Swanson & Cowan, 1979; Vinogradova, Kitchigina & Zenchenko, 1998; Bland, 1986). Oscillations in the theta range are generated intrinsically in cells within various limbic regions, and these oscillations then provide extrinsic influences via many reciprocal synaptic connections. Thus, the limbic cortex may be viewed as multiple synchronizing systems. The functional implications of this process are the entrainment of large subsets of neural circuitry in limbic structures into a common processing mode, turning them selectively for the reception of particular types of information flow. Since the understanding of the physiology and pharmacology of theta rhythms continues to be an important objective (Dickson, Kirk, Oddie, & Bland, 1995), further analysis of these theta-generating limbic structures is necessary.

HPC. By the 1960's it was concluded that the CA1 pyramidal region of the HPC was the main zone of theta generation since theta showed a phase reversal just below this area. Technical advancements in the early 1970's allowed subsequent researchers to examine extracellularly recorded neurons in freely moving animals. O'Keefe and

Dostrovsky's pioneering study in 1971 distinguished two classes of hippocampal formation neurons recorded during theta mode behaviors. Recording from CA1 hippocampal units, they named the first class of units *place units* because they had firing rates dependent on the animal's position on the testing platform. The second class of units was named *displace units* because their activity was related to the rat's motor behavior regardless of the animal's spatial orientation. Such progressive research led to the discovery of other HPC theta generators. Jonathon Winson was the first to report the finding of a second generator of theta located in the dentate region of the hippocampal formation of rats (Winson, 1974). This data indicated that the theta recorded from the CA1 and dentate generator regions was approximately 180 degrees out of phase. Additional research indicated that both rhythmic-bursting neurons of the CA1 region and the DG are modulated by the MSA.

MSA. Another major contribution at this time came from the "Vienna group" in the form of the medial septal cell pacemaker theory for hippocampal theta activity (e.g., Petsche, Stumpf & Gogolak, 1962). Studies of hippocampal unit discharges supported the conclusion that these medial septal pacemaker cells were providing input to CA1 pyramidal cells in the hippocampus proper (Green & Machne, 1955). Further studies indicated that this relationship is not reciprocal; namely that the rhythmicity of medial septal neurons is not dependent on afferent connections from the hippocampus (Alonso, Gaztelu, Buno, Garcia-Austt, 1987; Gaztelu & Buno, 1982). Previous studies have repeatedly demonstrated that disrupting MSA neuronal activity significantly suppresses theta rhythm in the HPC (Vinogradova, Kitchigina & Zenchenko, 1998; Givens, 1996; Andersen, Bland, Myhrer & Schwartkroin, 1979), entorhinal cortex (EC) (Mitchell,

Rawlins, Steward, & Olton, 1981; Partlo & Sainsbury, 1995), and raphe nucleus (Partlo & Sainsbury, 1995). Additional researchers have supported these findings (Vinogradova et al., 1998) and indicated that other subcortical structures including the MFB and DMH influence the MSA (Andersen et al, 1979; Destrade, 1982; Vinogradova, 1995; Vinogradova et al., 1998).

MFB and DMH. In his review of previous pharmacological and neurophysiological investigations in rats, Destrade (1982) suggested that the hippocampal afferents activated by medial forebrain bundle (MFB) stimulation must be different from those activated by dorsomedial hypothalamus (DMH) stimulation. For instance, a local injection of tetracaine in the medial septum, which reversibly interrupts the functional activity of the fornix (Andersen et al., 1979) suppresses the 6-8 Hz theta, but is without effect on the 8-12 Hz DMH-driven theta. The same injection in the cingulate cortex selectively blocks the high frequency theta (Destrade & Ott, a, 1980). In addition, the 8-12 Hz theta is probably mediated by a glutaminergic innervation of the HPC (Destrade & Ott, b, 1980). In other words, it is likely that the MFB-driven theta is generated from the classical fornix, whereas the DMH-driven theta is mediated via a different subcallosal route, reaching the perforant path which forms a glutaminergic input to the HPC (White, Nadler, Hamberger, Cotman & Cummins, 1977).

HPC electrographic patterns similar to the waves making up normal theta can be generated by appropriate electrical stimulation of specific septal, hypothalamic and brain stem sites. In this manner, Destrade (1982) has recently demonstrated in rats the possibility of driving, by diencephalic stimulation, two kinds of hippocampal theta, the appearance of which depended on the parameters of stimulation and the placement of

diencephalic electrodes. Stimulation of the DMH with current frequencies from 50 to

150 Hz and intensities from 25 to 200 µA elicited a large-amplitude hippocampal theta

with a frequency range of 8-12 Hz. Stimulation of the MFB with the same parameters

was accompanied by theta with maximum frequencies of 7-8 Hz (Destrade, 1982). Thus,

the physiological type of theta appears to determine how it is generated.

Theta Physiology

Types of theta: Type I. Theta can be pharmacologically separated into two types,

which differ in their sensitivity to muscarinic antagonists. The first is known as atropine

resistant theta (also called type I theta) and is abolished by anesthetic doses of urethane,

lesions of the entorhinal cortex (EC) and cingulum, or treatments that reduce forebrain

levels of serotonin. Type I theta is associated with a certain class of movements

including walking, running, swimming, rearing, jumping, digging, manipulation of

objects with the forelimbs, isolated movements of the head or one limb and shifts of

posture (Bland, 1986; Whishaw & Vanderwolf, 1973; Sainsbury, 1997). This theta has a

frequency range of about 7-12 Hz. It cannot be abolished by large doses of atropine

sulfate, but it is abolished by anesthetics such as ethyl ether, urethane and pentobarbital.

Therefore, it appears that type 1 theta is related to certain kinds of movement and is due

to activity in an ascending system that is atropine-resistant and anesthetic-sensitive.

In his examination of behavioral correlates of hippocampal field activity,

Sainsbury (1997) noted that a simple motor movement hypothesis for hippocampal theta

was insufficient. Complications with this theory appeared when observations were made

in rabbits that displayed long trains of theta during immobility (Harper, 1971; Klemm,

1971). Since these early reports, researchers have demonstrated a second type of theta activity in the rat and other animals (Bland, 1986).

Types of theta: Type II. The second is known as atropine sensitive theta (or type II theta) and is not affected by the above-mentioned treatments, but is sensitive to treatments that antagonize muscarinic neurotransmission (Dirkson et al., 1995). Type 2 theta is operationally defined as theta that occurs in the complete absence of movement. In his review of hippocampal theta, Bland (1986) states that rats produce short trains of type 2 theta prior to jump avoidance and during the presentation of stimuli associated with a conditioned emotional response. Type 2 theta in rats has a slightly lower overall frequency range of 4-9 Hz. Type 2 theta occurs during immobility and is resistant to most anesthetics but is sensitive to cholinergic antagonists such as atropine sulfate and scopolamine. Since type 2 theta can be elicited by eserine, this theta is probably a result of the activation of an ascending cholinergic pathway to the hippocampal formation.

Several lines of evidence suggest that the type 2 theta subsystem is always active when the type 1 theta subsystem is active (Destrade, 1982; Bland, 1986). This conclusion was reached by predicting that type 2 theta represents the involvement of the hippocampal formation in sensory processing. One line of evidence revealed that acetylcholine may be collected from the neocortex and hippocampus during both sensory stimulation and movement, suggesting that the cholinergic type 2 system is active at both times (Leranth & Frotscher, 1989). Further support of this conclusion resulted when researchers demonstrated that abolishing type 2 theta reduces type 1 theta. Specifically, this research demonstrated that abolishing theta cell rhythmicity (via administration of atropine sulfate) during type 2 theta conditions reduces the number of rhythmic

discharges during type 1 theta (Bland, 1986). While such extensive research into the anatomy and physiology of HPC theta rhythms has been conducted for decades, research into PFC theta has emerged only recently.

Prefrontal Cortex Theta

Groenewegen and Uylings (2000) reported that both the prefrontal cortex and the hippocampus are crucial for memory encoding and recall. However, it remains unclear how these brain regions communicate to exchange information. Recent findings using simultaneous recordings from the hippocampus and prefrontal cortex of the behaving rat have demonstrated that prefrontal cells' firing is phase-locked to the hippocampal theta rhythm. This suggests that phase synchronization clocked by the theta rhythm could be crucial for the communication between hippocampal and prefrontal regions (Jensen, 2005). To fully understand theta synchronization in connection with the PFC, an analysis of PFC anatomy is essential.

PFC anatomy. The prefrontal cortex (PFC) constitutes the cerebral cortical area in the frontal lobe rostral (front) to the motor and premotor cortices. Since this part of the cerebral cortex is not directly connected to any of the primary channels of sensory information nor directly linked to skeletomotor neurons, it has been difficult to functionally characterize the PFC. However, studies indicate that the PFC has a reciprocal relationship with two great functional realms, namely: (1) parietal and temporal regions of the cerebral cortex involved in the processing of visual, auditory and somatic sensory information, and (2) the telencephalic limbic system and its subcortical correspondents, in particular the hypothalamus and meso- and diencephalic structures associated with the hypothalamus (Groenewegen & Uylings, 2000).

The PFC directly projects to the cholinergic forebrain system and the monoaminergic cell groups in the hypothalamus and brain stem. In this way, the PFC is in a unique position to influence the transmitter systems that modulate large parts of the forebrain. Further, it has become clear that the PFC has a special relationship with the basal ganglia: the PFC not only projects via the cortico-striatal system to the basal ganglia but also receives projections from these structures via the thalamus. In this way, the PFC is involved in a number of largely parallel, functionally segregated cortical-subcortical circuits that subserve sensorimotor, cognitive, emotional/behavioral and visceral functions (Alexander, DeLong & Strick, 1986; Alexander, Crutcher & DeLong, 1990; Strick, Dum & Picard, 1995).

It was only after the introduction of the modern tracing studies that direct projections from the hippocampal formation to the PFC could be demonstrated in rats (Swanson, 1982). Later studies confirmed and extended these observations, showing that the CA1-region and the subiculum project to both the medial PFC (Jay & Witter, 1991) and the lateral PFC (Verwer, Meijer, Van Uum & Witter, 1997). The strongest contribution to the HPC-PFC projections originates from the ventral hippocampus. According to Groenewegen and Uylings (2000), an important conclusion is that limbic structures such as the HPC (and amygdala) not only have a profound influence on extensive areas of the PFC, but that they are intricately involved in the entire forebrain network in which the PFC constitutes the highest hierarchical level. Additional research supports this conclusion as the infralimbic and prelimbic cortices and the lateral PFC in the rat have reciprocal connections with the perirhinal and entorhinal cortex, and with the

CA1 field and subiculum of the hippocampal formation (Groenewegen, Wright & Uylings, 1997; Jay & Witter, 1991; Verwer et al., 1997).

Additional studies concerning the neural network among the PFC, HPC and theta rhythms were conducted by Asada, Fukuda, Tsunoda, Yamaguchi and Tonoike (1999). They reported that frontal midline theta rhythms (Fmθ) reflect alternative activation of the prefrontal cortex and anterior cingulated cortex (ACC). In their research, these regions were alternatively activated in about 40 to 120° phase shift during one Fmθ cycle. Results suggested that the appearance of Fmθ during consecutive mental tasks reflects alternative activities of the medial prefrontal cortex (mPFC) and ACC. When EEG recordings showed Fmθ containing a train of rhythmic bursts of 6-7 Hz, theta rhythms with the same frequency were simultaneously observed in frontal and temporal magnetoencephalogram (MEG) detectors (magnetic Fmθ: mFmθ).

The PFC and the limbic association cortex including the ACC are known to be mutually connected (Pandya & Seltzer, 1982). This connection in tandem with Asada et al.'s (1999) findings led to the proposal that the appearance of Fmθ rhythms during mental tasks is associated with interactions of both regions of the ACC and the prefrontal-medial superficial cortex and reflects a composite of potentials from them. Additional researchers have supported PFC neural network using anterograde transport studies (Gaykema, Van Weeghel, Hersh & Luiten, 1991). Such studies have revealed a topographic organization of the PFC, such that medial prefrontal areas project to the specific areas of the MSA. These studies have also discovered efferent projections from the PFC that clearly divert from the main corticofugal pathways to specifically innervate

the MSA (Gaykema et al., 1991). Therefore, as with the HPC, the MSA also has direct and reciprocal anatomical connections with the PFC.

Summary

In comparison to hippocampal theta rhythms, very little research has been conducted on prefrontal theta rhythms. For instance, it is uncertain how many types of theta the PFC contains and if they have the same properties as hippocampal theta rhythms. Furthermore, research is needed to conclude which anatomical structure(s) drive PFC theta. While this research has been repeatedly conducted with hippocampal theta rhythms, the same systematic approach to studying prefrontal theta rhythms has not yet been conducted. With the vast array of research indicating that theta rhythms are intimately involved with memory and motor behavior, PFC theta rhythms must be investigated. In this study, an analysis of PFC theta rhythms with and without cholinergic input from the MSA will be conducted.

Method

Subjects

Nine adult male Long-Evans rats, each weighing approximately 250 grams at the start of the experiment, were used. The rats were individually housed in 16.5in x 8in x 8in cages and maintained in a 12 hour light-dark cycle (lights on at 0700). All rats were placed on ad libitum food (LM485 Rat & Mouse Diet from Harlan Teklad) and water. All rats were fed, housed and treated under aseptic conditions according to the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, D.C., 1996)

and procedures approved by IWU's Institutional Animal Care and Use Committee (IACUC).

Procedure

Surgery. Rats were anesthetized prior to surgery via intraperitoneal injection of 0.1ml/kg ketamine/xylazine (8mg ketamine; 1.2mg xylazine). Supplemental injections of ketamine/xylazine were administered if corneal, hindlimb, tail reflexes, excessive whisker movement or rapid respiratory rates became present. The initial supplemental injection, if needed, had a concentration of 0.09ml/kg ketamine/xylazine (7.2mg ketamine; 1.08mg xylazine) and all further supplements were concentrated at 0.07ml/kg ketamine/xylazine (5.6mg ketamine; 0.84mg xylazine). All surgeries took place in the afternoon, so to increase the efficacy of the anesthesia, rats were not fed in the morning before surgery.

After the rats are anesthetized, a sterile White Petrolatum Mineral Oil and Lanolin Oil lubricant was placed on their eyes to prevent irritation and dryness. Following eye ointment application, the rats' heads were shaved and secured into a stereotaxic device for immobilization. Heating pads were placed under the rats to prevent the loss of body heat that accompanies anesthetization. Rats' scalps were cleansed with betadine scrub before the opening incision and sterile, autoclaved cotton-tip applicators were used when clearing blood and debris following the opening incision. After the opening incision exposed the skull, surgical clips were attached to the faschia to hold the skin back. After the skull was exposed, holes were drilled and an A-M Systems, Inc. (Sequim, WA) ground wire (0.010in bare; 0.0130in coated) with a gold ITT/Cannon Centi-Lok pin attached to one end was lowered into the parietal cortex (4.0 mm posterior to bregma; 2.5

mm lateral to midline; 1.5 mm ventral to the dural surface). A stainless steel Teflon-coated recording electrode (0.005in bare; 0.008in coated), also with a gold pin at one end, was implanted unilaterally into the prefrontal cortex (0.6 mm anterior to bregma; 1.5 mm lateral to midline; approximately 3.75 mm ventral to the dural surface). A Plastics One (Roanoke, VA) C315GA 26GA acute guide cannula was lowered at a 15 degree angle into the medial septal area (MSA) (0.8 mm anterior to bregma; at the midline; 3.8 mm ventral to the dural surface).

After the ground wire, recording electrode and guide cannula were inserted, Plastics One (Roanoke, VA) dental cement was used to solidify the components in place. Prior to applying the dental cement, 2-4 S/S MACH 1/8 inch screws (Small Parts, Inc.) were inserted into the skull to provide extra adhering surfaces for the cement. The recording electrode and ground wire were then inserted into an ITT insulator to prevent damage to the wires. The ITT insulator was then affixed with dental cement. Once the dental cement hardened, rats were removed from the stereotaxic device and given an i.p. injection of Rimadyl (0.2mg/kg), a non steriod anti-inflammatory drug, to reduce discomfort. Mycitracin Plus, a local antibiotic/anesthetic, was then applied to the edges of the dental acrylic to prevent infection and minimize discomfort. The rats resumed free-feeding post-surgery and body weight was measured everyday for the remainder of the experiment to ensure the continued health of the rats.

Post-Surgical Recording. Theta electroencephalographic (EEG) recordings took place two weeks post-surgery. Each rat was recorded twice with a minimum three day delay between sessions to ensure any previous anesthesia effects were eliminated. Rats were anesthetized with an i.p. injection of .1ml/kg ketamine/xylazine (8mg ketamine;

1.2mg xylazine) at the beginning of the recording sessions, all of which were in the afternoon. For increased anesthesia efficacy, rats were not fed the morning of their afternoon recording sessions. To collect the electrophysiological data, a preamplifier and cable were connected to the rat's head. The signal was passed to an amplifier (1,000 x z)/filter (0.1-500 Hz) system (A-M Systems, Inc.), which in turn sent the signal to an A-D board for digitization. The digitized signal was sent to data acquisition software developed by DataWave Systems (Longmont, CO). After a five minute baseline EEG recording, the rats received a counterbalanced drug infusion of scopolamine (10μg dose), a cholinergic receptor antagonist, or saline into the MSA as outlined above. Half of the rats received scopolamine infusions on the first recording day and received saline infusions on the second recording day and vice versa. A total of 0.5 μl of scopolamine/saline was administered into the MSA at 0.2 μl/min over a 2.5 minute span. The stylet was then removed from the guide cannula in the MSA and replaced with a sterile injector, followed by a 5 minute wait post-infusion to allow the scopolamine to take effect. The theta rhythm in the prefrontal cortex was then recorded for 10 minutes post-infusion. After recording, the stylet was reinserted into the guide cannula and rats were individually placed back in their cages.

Electrophysiological Data. All electrophysiological data was analyzed using software developed by Biopac Systems, Inc. All electrophysiological records were examined for noise and all non-neural signals were removed before being further examined for theta activity. To determine whether PFC theta activity was dependent on the MSA, a quantitative analysis was applied to the electrophysiology records. A fast fourier transformation (FFT) analysis was performed on both the baseline block and drug

(saline/scopolamine) block to determine theta power (amplitude) and frequency. The FFT results were subjected to a within-subjects analysis of variance (ANOVA) using drug condition as a within subject factor and theta power and frequency as dependent variables.

Histology. Histological verification of the component placements and drug effects are forthcoming. To verify the accuracy of the component placements and to determine the extent of the drug infusions, the rats will be deeply anesthetized with sodium pentobarbital. An electrical current (20 μA for 40 s) will then be passed through the electrode to produce a lesion at the tip of the recording and ground electrodes followed by an infusion with Chicago Sky blue dye (2%; 0.5 μl) into the MSA to verify the location of the medial septal guide cannula placement. Following the dye infusions and electrolytic lesions, each rat will be perfused transcardially first with saline (200 ml) and then with a 10% formalin solution (200 ml). The brains will be sectioned (40 μm), mounted on gelatin coated slides and stained with cresyl violet. Lesion sites will then be examined with a light microscope to determine placement accuracy.

Results

Of the nine rats used in the study, three of the rats died post-surgery and data collection is currently ongoing with two rats. Therefore, the following analyses were conducted on four subjects.

A quantitative analysis using a fast fourier transformation (FFT) was applied to the electrophysiology records to determine theta power (amplitude) and frequency. Results were subjected to a within-subjects analysis of variance (ANOVA) using drug

condition as the within subjects factor and theta power and frequency as dependent variables. The drug condition was comprised of four measures: saline baseline, saline, scopolamine baseline, and scopolamine.

Theta Power. The one way repeated measures ANOVA which analyzed the measure of drug condition indicated a main effect for theta power, $F(3,9) = 4.06$, $p < .05$. Three paired samples t-tests were then conducted for theta power differences between the drug condition measures. Analyses to examine whether PFC theta power was dependent upon the MSA revealed that scopolamine infusions into the MSA significantly decreased theta power, with significant effects being observed between saline power and scopolamine power ($t(3) = 3.729$, $p = .034$; Fig. 3) and scopolamine baseline power and scopolamine power ($t(3) = 4.101$, $p = .026$; Fig. 4). These effects could not be explained as simple time effects (i.e., time after anesthesia), as no significant difference was observed between saline baseline power and saline power, $t(3) = 1.209$, $p = .313$; Fig. 4. In addition, these effects were not simply due to differences between the day of infusion, as a comparison of saline baseline power and scopolamine baseline power was not significant, $t(3) = .911$, $p = .429$; Fig. 5.

Theta Frequency. The one way repeated measures ANOVA which analyzed the measure of drug condition indicated no main effect for theta frequency, $F(3,9) = .696$, $p > .05$. Unlike the results for theta power, neither saline nor scopolamine infusions into the MSA had a significant effect on theta frequency (Figs. 6-8).

Discussion

These results provide the first demonstration that delivery of the muscarinic receptor antagonist scopolamine into the MSA significantly decreases theta power in the PFC, confirming our hypothesis that PFC theta power is dependent on the MSA. However, future studies will need to examine the anatomical pathway(s) by which the MSA is affecting PFC theta. While disrupting the MSA could directly affect the PFC, it is also possible that the MSA is indirectly modulating PFC theta through other brain structures, such as the HPC. It is also possible that additional neurotransmitter systems besides acetylcholine are affecting theta in the PFC. Therefore, further investigation into the direct and indirect anatomical pathways and neurotransmitter systems potentially involved in MSA control of PFC theta is necessary.

Direct MSA Anatomical Pathways

    MSA as a widespread theta generator. When combined with previous studies of the anatomical substrates of theta EEG activity, these results implicate the MSA as a region responsible for controlling theta generation in many neural networks. Indeed, the MSA is part of the basal forebrain which contains magnocellular cholinergic neurons which serve as the major source of cholinergic innervation of many brain structures (Gaykema et al., 1991), including the cerebral cortex, HPC, amygdala and olfactory bulb. In the rodent brain, groups of these neurons are present in the MS and nuclei of the diagonal band of Broca (DB), forming the rostral (front) portion of the basal forebrain cholinergic system.

    Previous studies have repeatedly demonstrated that disrupting MSA neuronal activity significantly suppresses theta rhythm in the HPC (Vinogradova et al., 1998; Givens, 1996; Andersen, Bland, Myhrer & Schwartkroin, 1979), entorhinal cortex (EC)

(Mitchell et al., 1981; Partlo & Sainsbury, 1995), and raphe nucleus (Partlo & Sainsbury, 1995). The present study adds to these results by demonstrating that disrupting MSA neuronal activity through scopolamine infusions also significantly suppresses theta power in the PFC. Unpublished results from our laboratory further indicate that disrupting MSA neuronal activity suppresses theta rhythm in the amygdala as well. Such results suggest that the MSA exerts significant control over the theta rhythm in every theta-generating brain structure it has connections with. In support of the hypothesis that PFC theta is directly controlled by the MSA, examination of MSA-HPC and MSA-PFC interactivity yields many similarities.

MSA control of HPC theta. Numerous studies have revealed that the MSA has direct anatomical connections with the HPC (Risold & Swanson, 1996; Swanson & Cowan, 1979; Swanson, 1982). The medial division of the septal region (MS-DB) projects onto the HPC via the septo-hippocampal pathway, or fornix (Daitz & Powell, 1954). Reciprocally, the HPC projects to the lateral septal nucleus (Swanson & Cowan, 1977), which in turn projects to the medial septal nucleus (MS) and nucleus of the diagonal band (DB) (Raisman, Cowan & Powell, 1966). In addition to anatomical connections, additional factors have led researchers to suggest that the MSA exerts direct control over HPC theta.

Lesion studies using a variety of species have repeatedly demonstrated that lesioning the MSA or the fornix results in the irreversible block of EEG theta generation in the HPC (Green & Arduini, 1954; Shaban, 1970). Additionally, the MSA contains neurons that naturally and regularly exhibit rhythmic bursting activity within the theta frequency range (Wilson, Moffer & Lindsley, 1976). When combined with the repeated

statistical finding that a strong positive correlation exists between the burst activity of MSA neurons and HPC theta activity, these findings implicate the MSA as the "pacemaker" of HPC theta (Petsche & Stumpf, 1960). Thus, the rhythmically bursting neurons of the MSA are essential for HPC theta generation.

Additional research indicates that the "pacemaker" effect of the MSA directly results from a relatively small (6-8%) number of its neurons (Vinogrdova et al., 1998). By blocking MSA synaptic transmission using a medium of high magnesium-low calcium content, Belousov et al. (1979) and Vinogradova et al. (1998) showed that a majority (up to 80%) of previously bursting neurons started firing irregularly while 6-8% retained their regular bursting activity. Thus, these neurons are regarded as real bursting pacemakers whereas the other MSA neurons are regular pacemakers that are secondarily involved in rhythmic bursting activity by synaptic influences. While the rhythmically bursting effect of the MSA is therefore due to real bursting pacemaker neurons, additional research indicates the frequency of these neurons is controlled by connections to the MSA.

Studies have revealed that there are two ways of increasing the power and synchrony of HPC theta (Vinogradova, 1998; Brazhnik & Vinogradova, 1986; Monmaur, Collet, Puma, Kohn & Sharif, 1996): stimulating the brain stem (extraseptal) and increasing levels of MSA acetylcholine (intraseptal). However, important to note from these studies is that while both intraseptal and extraseptal manipulations affect theta power, only extraseptal manipulations impact theta frequency. Overall, these patterns of HPC theta EEG activity are also apparent when examining PFC theta activity.

MSA control of PFC theta. There are anatomical and physiological similarities between HPC and PFC theta. Anatomically, as with the HPC, the MSA also has direct and reciprocal connections with the PFC. Anterograde transport studies have revealed a topographic organization of the PFC, such that medial prefrontal areas project to the MS, VDB, and medial part of the HDB, whereas the orbital and agranular insular areas predominately innervate the HDB and MBN, respectively (Gaykema et al., 1991). Such studies have also discovered efferent projections from the PFC that clearly divert from the main corticofugal pathways to specifically innervate the MSA (Gaykema et al., 1991). Such reciprocal anatomical connections between the MSA and PFC parallel those between the MSA and HPC, suggesting that the MSA might exert a similar effect on PFC theta generation as it does on HPC theta generation.

Indeed, our results strongly support this suggestion, as temporarily inhibiting the MSA-PFC cholinergic pathway resulted in the same decrease in theta power observed by other researchers (Monmaur et al., 1996; Vinogradova, 1998; Brazhnik & Vinogradova, 1986) following inhibition of the MSA-HPC cholinergic pathway. Additionally, the fact that we did not observe any PFC theta frequency changes following MSA inhibition coincides with many previous studies of MSA-HPC theta (Vinogradova, 1998; Brazhnik & Vinogradova, 1986), as intraseptal manipulations traditionally affect theta power but not frequency. Thus, it appears that the MSA affects PFC theta in the same manner as HPC theta with regards to both power and frequency. However, given that the MSA, PFC and HPC innervate and are innervated by multiple areas of the brain, it is possible that the MSA controls PFC theta indirectly.

Indirect MSA anatomical pathways

While our results suggest that the MSA directly controls PFC theta, it is possible that the MSA is controlling or merely modulating PFC theta via other brain structures, such as the HPC. Thus, alternate hypotheses implicating the MSA as indirectly controlling PFC theta generation cannot be discarded. In this indirect model, the MSA could control HPC theta which in turn modulates PFC theta. Both anatomical and electrophysiological evidence suggest this might be a possibility.

Anatomical evidence. Functional interactions between the HPC and PFC are supported by anatomical data, as a direct projection from the HPC to the PFC has been demonstrated in several species (Goldman-Rakic, Selemon & Schwartz, 1984; Ferino, Thierry & Glowinski, 1987; Jay & Witter, 1991). In rats (Jay, Glowinski & Thierry, 1989; Jay & Witter, 1991) and cats (Anderson et al., 1977), restricted portions of the temporal HPC (subiculum and CA1) project to the prelimbic PFC. In addition to this excitatory HPC-PFC pathway, the profoundly interconnected nature of the PFC suggests that multiple brain structures may be influencing it.

Research provides evidence that an anatomical substrate of a direct and topographically differentiated PFC may influence basal forebrain regions and, in particular, their cholinergic neurons. Part of the PFC control on the cholinergic cells in the basal forebrain, and thus on the recurrent cholinergic projection to the cerebral cortex, HPC, olfactory bulb and amygdala, may be exerted through a direct monosynaptic loop (Gaykema, 1991). Indeed, data on neurotransmitter systems together with corticocortical systems demonstrate the unique (integrating and/or gating) position of the PFC (Uylings

& van Eden, 1990). Thus, it is reasonable to suggest that the MSA is not the only structure affecting neuronal activity in the PFC.

Electrophysiological evidence. Further studies of the HPC-PFC connection have suggested that HPC cells can induce long term potentiation (LTP) in the prefrontal cortex (Laroche, Jay & Thierry, 1990; Doyere, Burette, Negro & Laroche, 1993). LTP is manifested as a long-lasting increase in synaptic efficacy following high frequency electrical stimulation (Rose & Dunwiddie, 1986). Neurons connected by a synapse that has undergone LTP have a tendency to be active simultaneously (Whishaw & Vanderwolf, 1973); thus, after a synapse has undergone LTP, subsequent stimuli applied to the presynaptic cell are more likely to elicit action potentials in the postsynaptic cell (Klimesch, Doppelmayr, Yonelinas, Kroll, Lazzara, Rohm & Gruber, 2001). Such findings emphasize the importance of HPC theta rhythm in synchronizing the activation of HPC-PFC networks (Miller, 1991). However, despite the HPC's influence on the PFC through LTP, neither this nor the multiple afferent and efferent connections of the PFC definitively indicate the mechanism(s) by which PFC theta is controlled.

To test the hypothesis that the MSA controls PFC theta power indirectly, additional studies measuring multiple structures could be conducted. For instance, studies could measure the effect of MSA inhibition via scopolamine on PFC theta both before and after lesioning the HPC. Such a study would indicate whether the HPC is involved in generating PFC theta power. Additional studies could also observe the effect of brainstem lesions on MSA, HPC and PFC theta power and frequency. While such studies would contribute to our understanding of the structure(s) controlling theta, the fact that such structures may contain various types of neurotransmitters complicates this

issue. Although numerous neurotransmitter systems may play a role in MSA control of HPC and PFC theta, perhaps the two most widely implicated are the cholinergic and GABAergic neurotransmitter systems.

Neurotransmitter systems

Acetylcholine (ACh). In addition to its rhythmically bursting neurons, another widely accepted feature of the MSA is its significant population of neurons that project to the HPC and release the neurotransmitter ACh (Givens & Olton, 1990; Wu, Shanabrough, Leranth & Alreja, 2000). Cholinergic mechanisms operating within the MSA have proven to be critical for learning and memory (Wu et al., 2000), as drug infusions that excite ACh activity (muscarinic agonists) elicit continuous HPC theta (Monmaur & Breton, 1991) while ACh-inhibiting drugs (muscarinic antagonists) abolish HPC theta (Powell, 1979).

Studies indicate that destruction of the septal nuclei (and thus ACh neurons) produces significant decreases in cholinergic activity of the HPC (Asaka, Seager, Griffin & Berry, 2000). This, in turn, results in significantly reduced theta and subsequent impairment in learning and memory processes (Wu et al., 2000). Additionally, stimulation of the MSA increases the release of ACh from the HPC. Modulating neurotransmitter release by stimulating or inhibiting structures such as the MSA may be conducted through injections of agonists and antagonists. Studies using animal models have demonstrated that systematically administered muscarinic acetylcholine receptor antagonists produce learning impairments in various species (Powell, 1979; Asaka et al., 2000). Additionally, some studies have demonstrated that direct infusion of scopolamine,

a nonselective competitive inhibitor of muscarinic receptors, into the MSA produces

similar learning impairments (Givens & Olton, 1990).

As a cholinergic antagonist, scopolamine blocks acetylcholine from binding to

receptors. Therefore, when administered into the MSA, scopolamine blocks theta

modulation in the majority of HPC cells and renders their activity significantly

heterogeneous (Vinogradova, Brazhnik, Kichigina & Stafekhina, 1996). Scopolamine

further induces lengthening of the initial period of inhibition, or theta reset. In contrast,

the cholinergic agonist eserine stimulates ACh release and binding which effectively

alters the baseline activity of HPC neurons. This, in turn, induces an intensification of

theta modulation with an equally probable decrease or increase in the level of

spontaneous activity in different neurons (Vinogradova et al., 1996). Such research

suggests that cholinergic neurons (which secrete ACh) are excitatory rather than

inhibitory.

However, there is some controversy as to whether the cholinergic septal cells are

excitatory or inhibitory (Buzsaki, 1984). The assumed excitatory action of cholinergic

septal neurons on the HPC has been criticized due to research suggesting that septal cells

inhibit the HPC via direct excitation of inhibitory interneurons (Buzsaki, 1984). The fact

that the MSA contains both neurons that release acetylcholine and GABA (inhibitory)

neurotransmitters adds support to this hypothesis.

γ-aminobutyric acid (GABA). In evaluating anticholinergic drug effects on the

MSA, it is important to note that GABAergic as well as cholinergic projection neurons in

the MSA express muscarinic acetylcholine receptors (Asaka et al., 2000). Thus, it is

possible that antimuscarinic drugs, such as scopolamine, exert their effect on the HPC

through receptors on both GABAergic and cholinergic neurons in the MSA.

Immunocytochemical staining studies indicate that the density of muscarinic ACh

receptors were higher on GABAergic than on cholinergic neurons in the MSA (Van der

Zee & Luiten, 1994). These findings suggest that the direct infusion of scopolamine into

the MSA blocks muscarinic receptors on both GABAergic and cholinergic projection

neurons with perhaps stronger effects on GABAergic neurons (Asaka et al., 2000).

The argument for involvement of the GABAergic projection is further supported

by recent research showing that cholinergic neurons in the fornix were not excited by

muscarine, whereas muscarine produced a very strong excitatory effect on fornix

GABAergic neurons (Wu et al., 2000). By fluorescently labeling cholinergic neurons in

the fornix, these researchers have suggested muscarinic agonists (eserine) do not excite

but rather inhibit these neurons (Wu et al., 2000; Buzsaki, 1984). Thus, some researchers

have proposed that the increased HPC theta power and synchrony observed after injecting

muscarinic agonists into the MSA is not due to increased levels of ACh in the HPC

(Buzsaki, 1984). Rather, disinhibitory mechanisms caused by greater impulses in the

GABAergic neurons of the fornix may underlie the increased HPC theta power and

synchrony observed after injecting muscarinic agonists into the MSA (Wu et al., 2000).

Thus, the notion that MSA cholinergic neurons are excited by their own neurotransmitter

is debatable.

Conclusion

While this study is still in progress and more animals will need to be included, the

preliminary results indicate that the MSA controls PFC theta power. However, the study

raises two very important future considerations: how is the MSA controlling PFC theta

power (directly or indirectly) and by what neurochemical means (ACh and/or GABA

neurons). To address whether the MSA directly controls PFC theta, studies could

measure the effect of inhibiting the MSA on PFC theta both before and after lesioning the

HPC, thus indicating the effect (if any) of the HPC on PFC theta. To address cholinergic

versus GABAergic contributions to PFC theta, future studies might selectively lesion

ACh neurons via IGG 192-Saporin in the MSA and observe the subsequent effects on

PFC theta. In addition, a more extensive examination of PFC theta including

comparisons of type I versus type II theta, the effects of brainstem stimulation, and

alcohol effects would greatly contribute to the current understanding of PFC theta.

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Figures

Figure 1. Placement of recording electrode and guide cannula. Both the recording electrode and guide cannula were inserted at 15 degree angles into the anterior cingulate of the prefrontal cortex and medial septal area, respectively.

Figure 2. EEG recordings of PFC theta following saline and scopolamine infusions into the MSA. Whereas saline produced no significant effect on PFC theta power, scopolamine produced a significant decrease in PFC theta power. Neither infusion resulted in a significant change in PFC theta frequency.

Figure 3. Bar graph representation of PFC theta power after injections of saline and scopolamine into the MSA. Whereas saline produced no significant effect on PFC theta power, scopolamine produced a significant decrease in PFC theta power. *$p < .05$.

Figure 4. Comparison of baseline conditions and drug effects for saline and scopolamine on PFC theta power. No significant difference in PFC theta power was observed between the saline baseline condition and infusion of saline. A significant difference was observed between the scopolamine baseline condition and infusion of scopolamine, indicating that the significantly decreased PFC theta power was due to scopolamine and not time effects (changes in theta merely due to time after receiving anesthesia). * $p < .05$.

Figure 5. Comparison of baseline conditions for saline and scopolamine for PFC theta power. Since subjects received counterbalanced drug infusions on different days, this graph indicates that there was no significant difference in PFC theta power between recording sessions.

Figure 6. Bar graph representation of PFC theta frequency after injections of saline and

scopolamine into the MSA. Neither saline nor scopolamine produced a

significant effect on PFC theta frequency.

Figure 7. Comparison of baseline conditions and drug effects for saline and scopolamine

on PFC theta frequency. No significant difference in PFC theta frequency was

observed between either the saline baseline condition and infusion of saline or

scopolamine baseline condition and infusion of scopolamine, indicating that

theta frequency was unaffected by time effects (changes in theta merely due to

time after receiving anesthesia).

Figure 8. Comparison of baseline conditions for saline and scopolamine for PFC theta

frequency. Since subjects received counterbalanced drug infusions on different

days, this graph indicates that there was no significant difference in PFC theta

frequency between recording sessions.
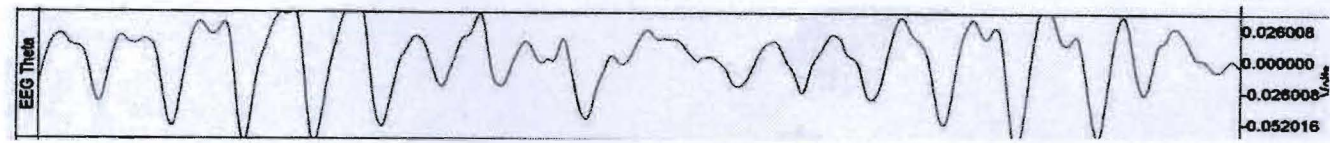
Figure 1.

Figure 2.

Prefrontal theta rhythm following saline infusion
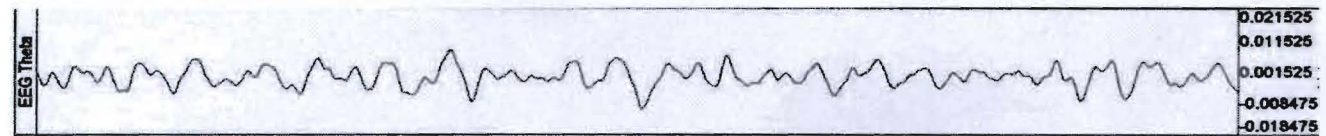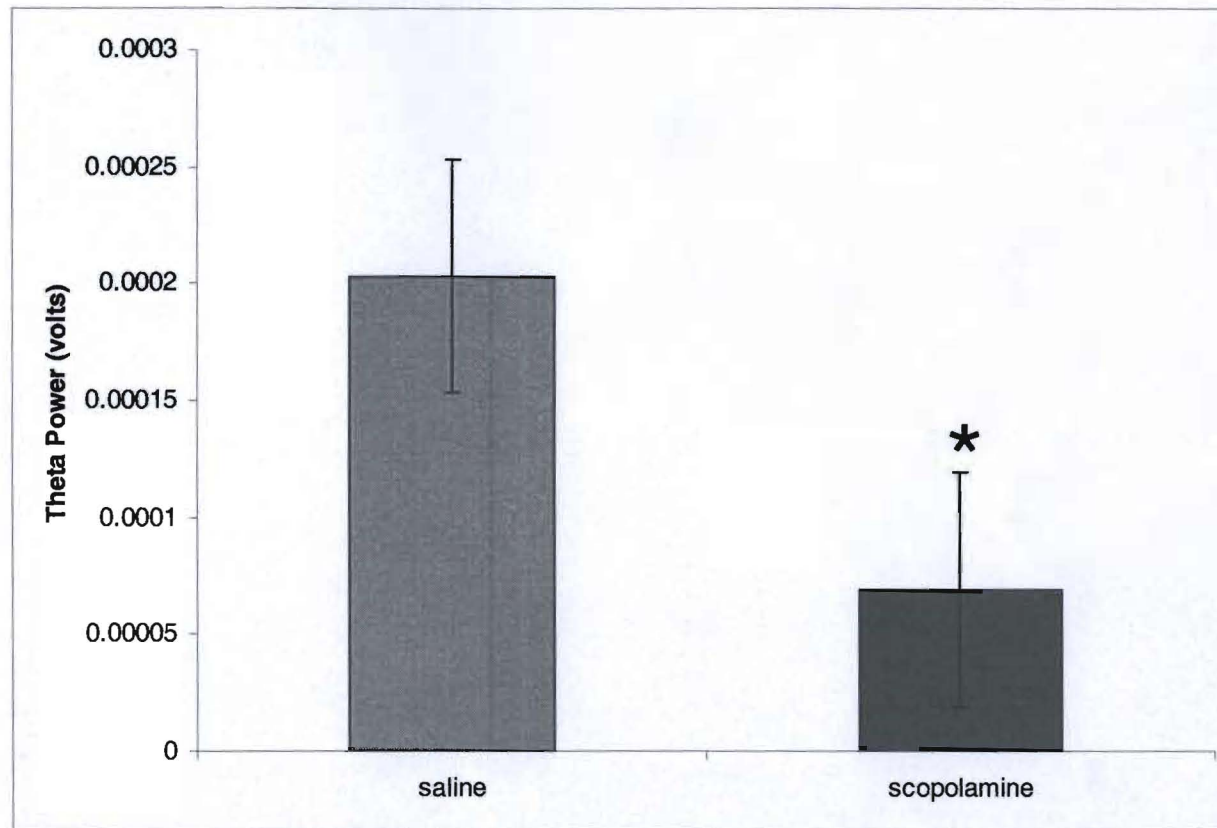

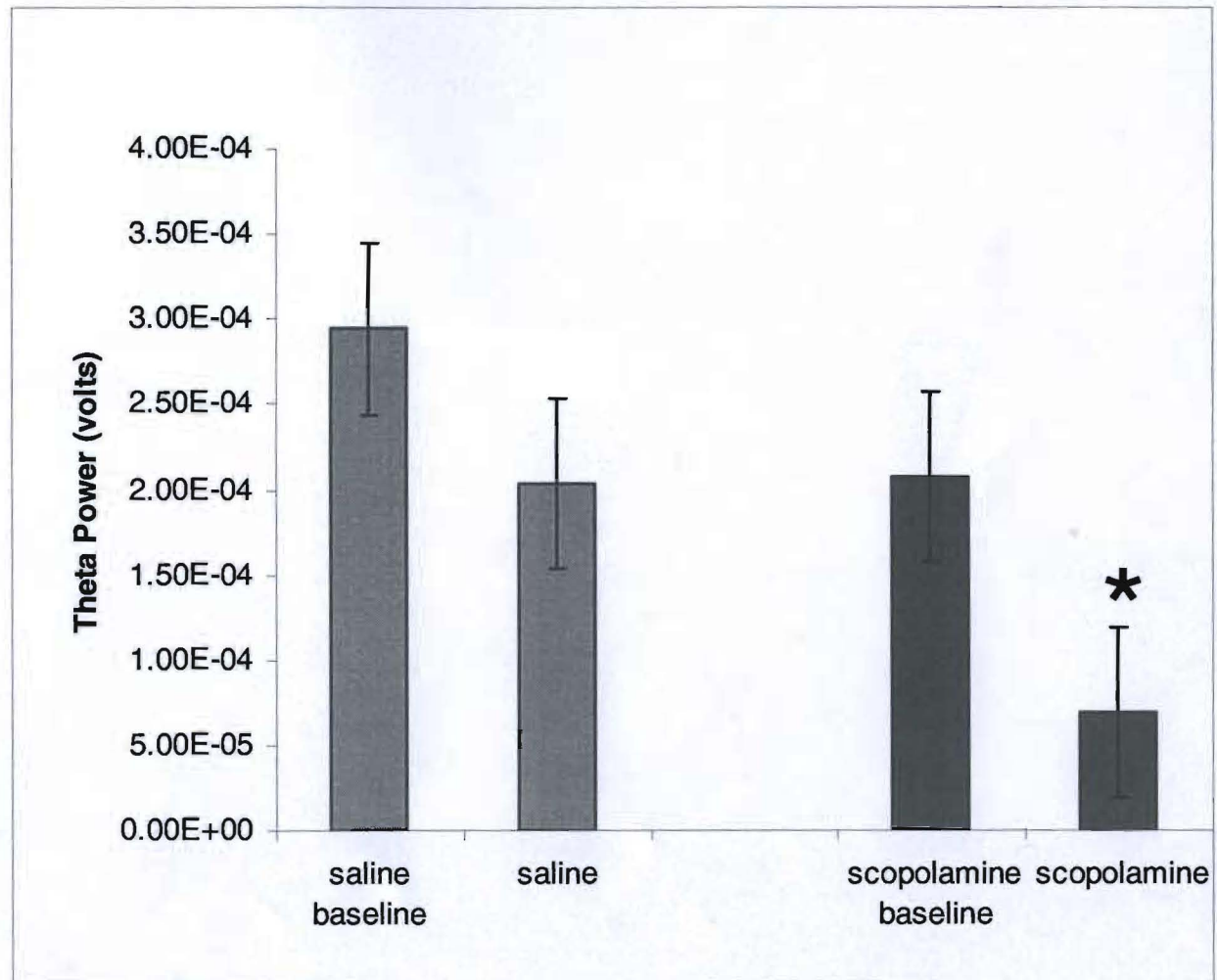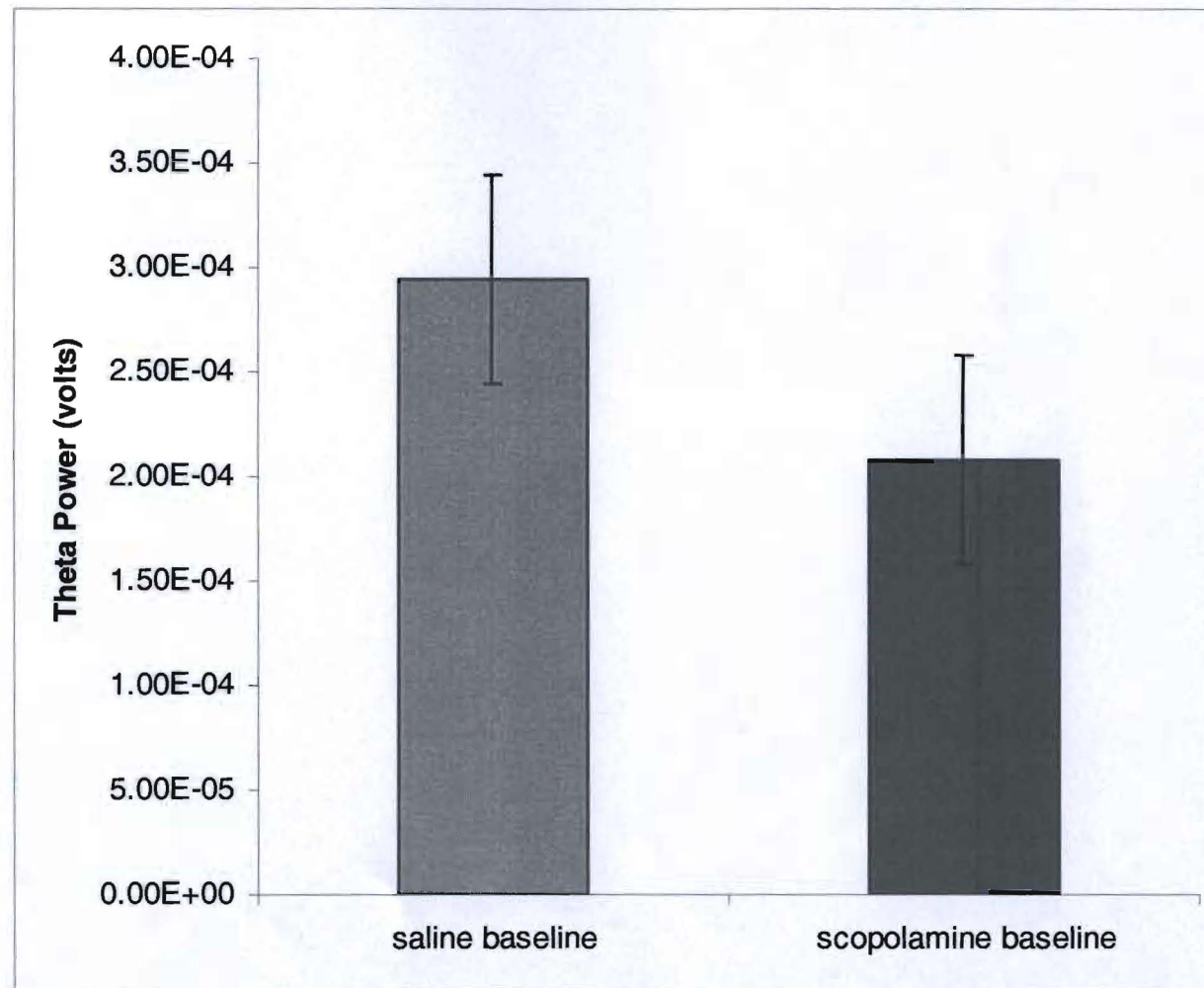Prefrontal theta rhythm following scopolamine infusion
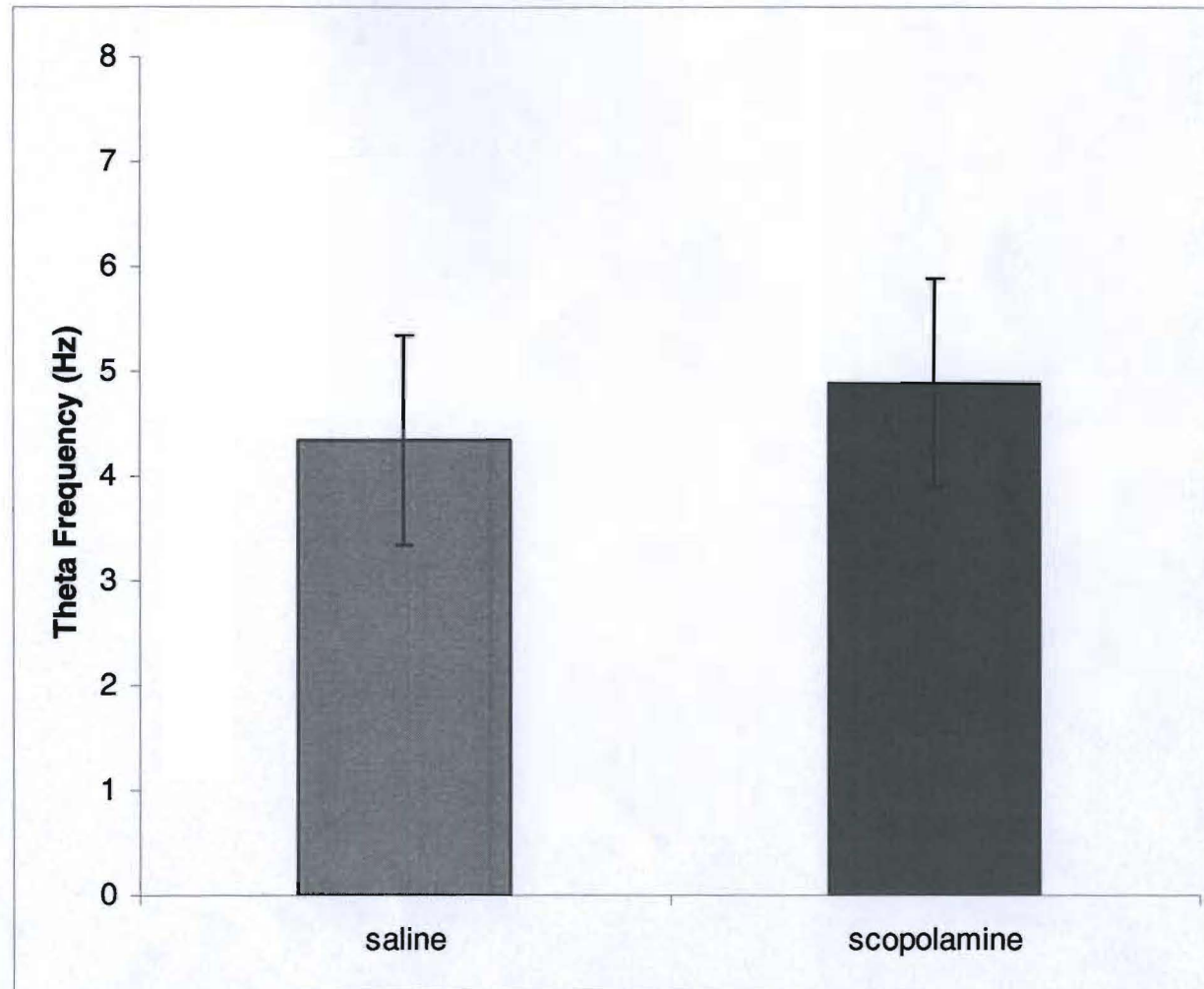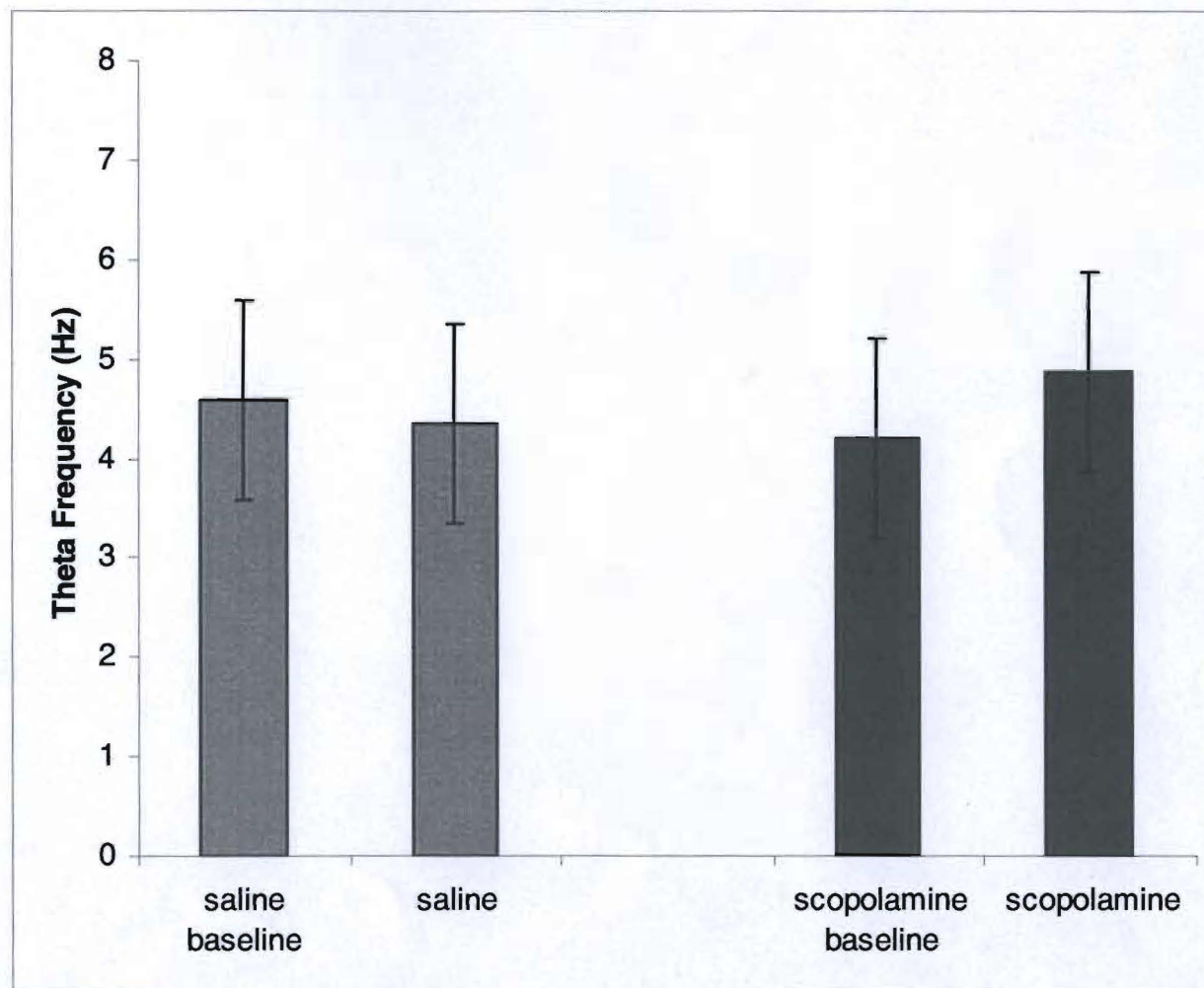
Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.